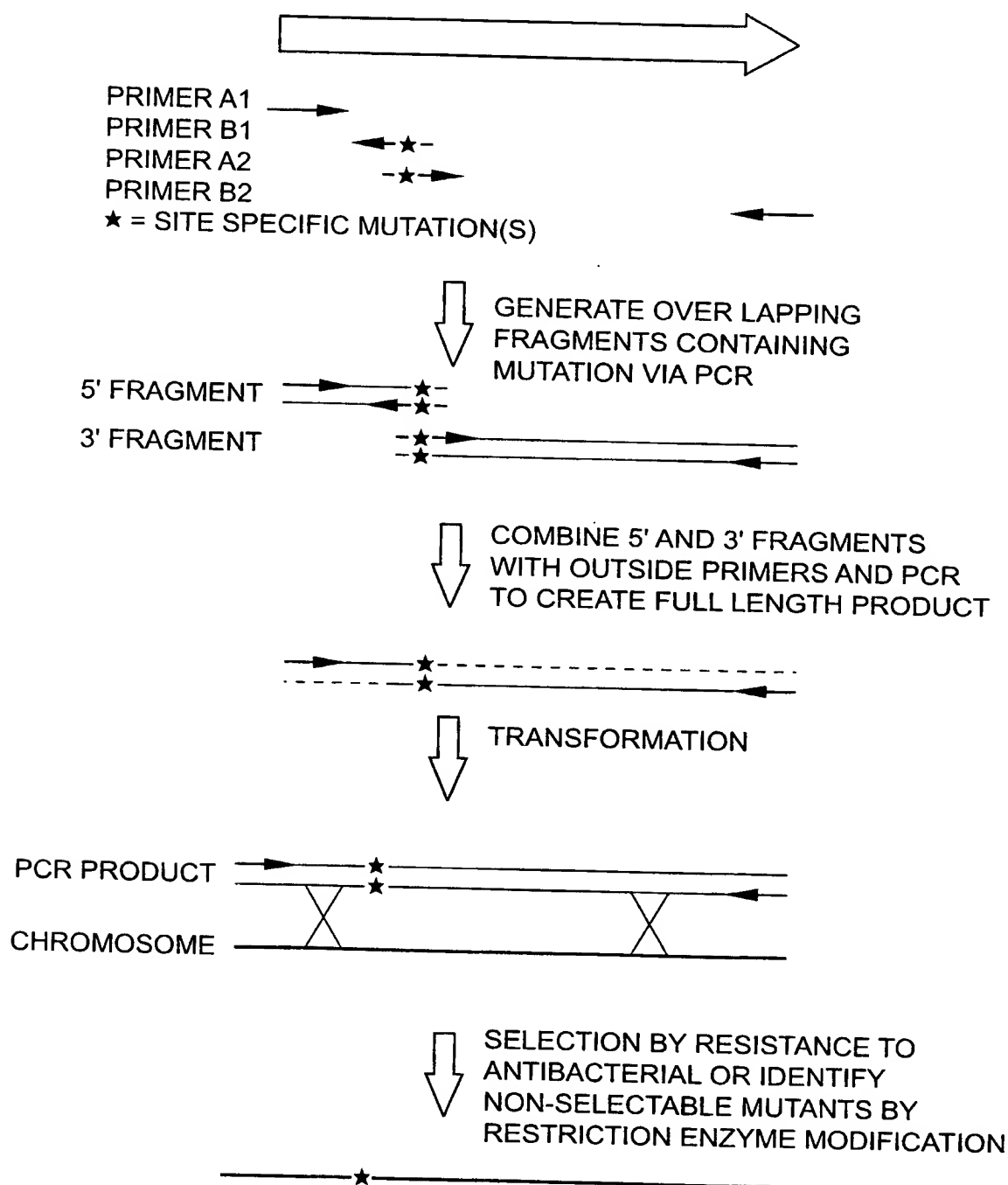
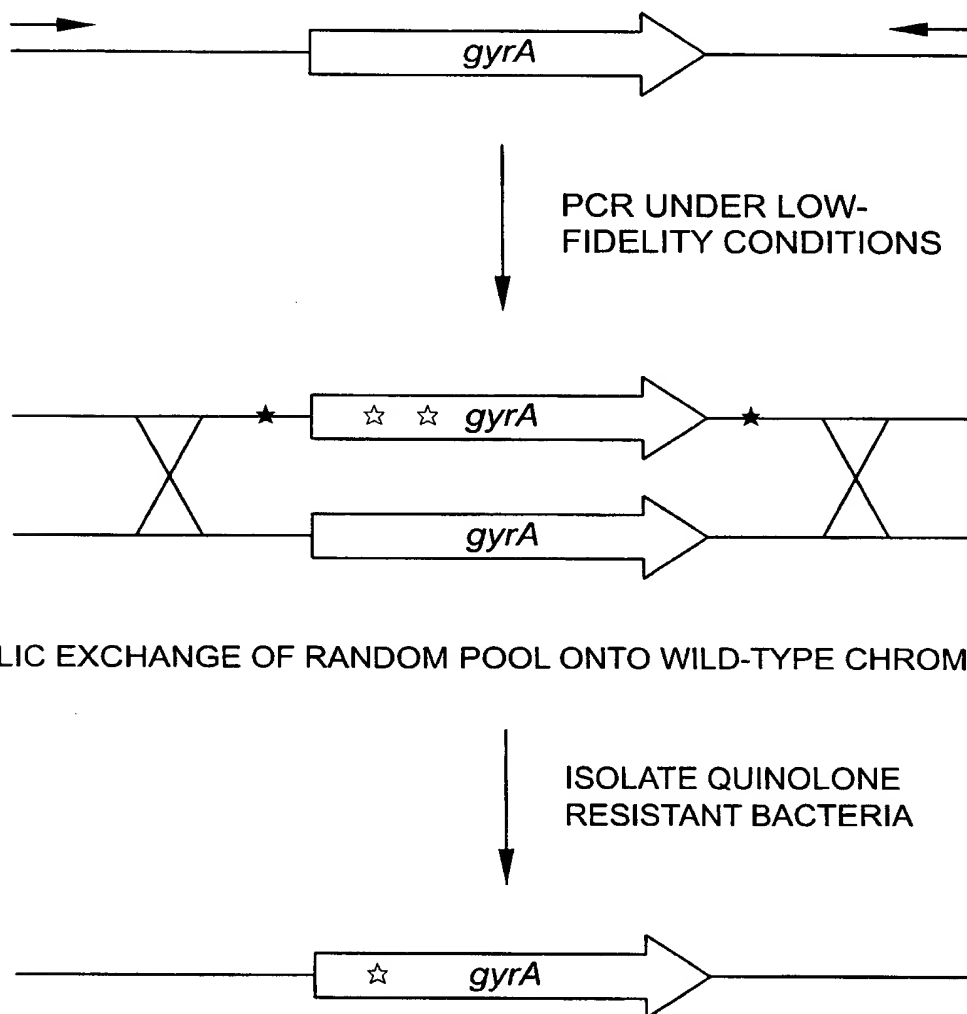


# FIG-1 RAPID GENERATION OF SITE-SPECIFIC CHROMOSOMAL MUTANTS



**FIG-2** RANDOM MUTAGENESIS AND IDENTIFICATION  
OF MUTATIONS RESPONSIBLE FOR  
QUINOLONE RESISTANCE



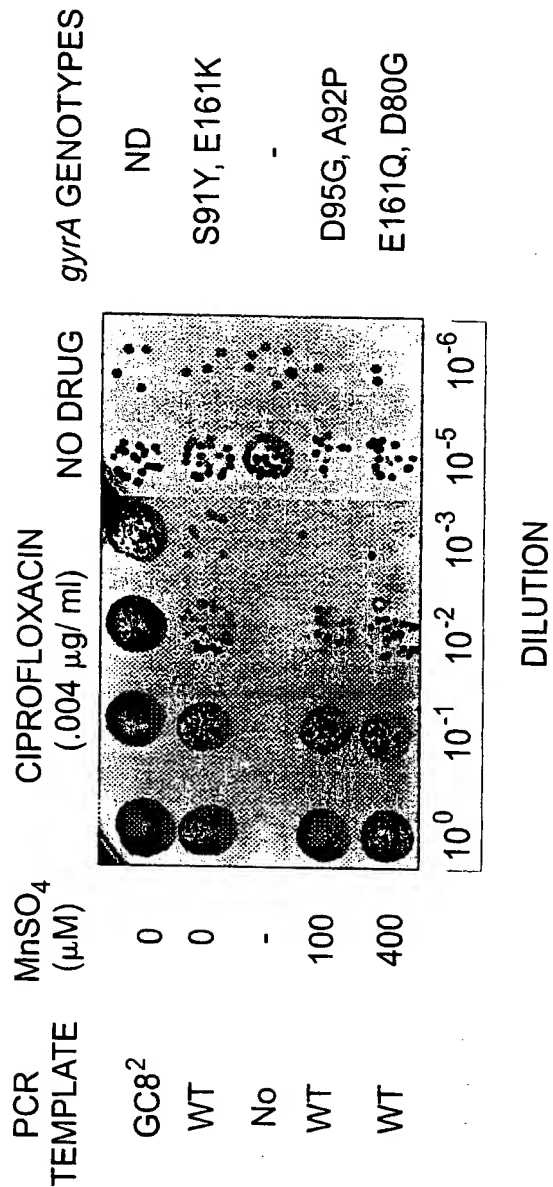
ALLELIC EXCHANGE OF RANDOM POOL ONTO WILD-TYPE CHROMOSOME

ISOLATE QUINOLONE  
RESISTANT BACTERIA

SEQUENCE RESISTANT ALLELE TO IDENTIFY MUTATION

002227 29957260

FIG-3



# FIG-4 RAPID ANTIMICROBIAL TARGET ELUCIDATION (RATE)

1) GENERATE A LIBRARY OF RANDOM CHROMOSOMAL POINT MUTATIONS BY PCRing WITH LOW-FIDELITY CONDITIONS

2) TRANSFORM POOLS OF 12 PCR PRODUCTS (100kB) INTO A WILD-TYPE BACKGROUND AND ISOLATE STRAINS RESISTANT COMPOUND

3) RE-TRANSFORM WITH INDIVIDUAL PRODUCTS (10kB) FROM POOLS WHICH CONFERRED RESISTANCE TO IDENTIFY FRAGMENTS WITH MUTATION(S)

4) GENERATE SMALLER PCR PRODUCTS (1kB) TO FURTHER MAP MUTATION(S) RESPONSIBLE FOR PHENOTYPE

5) SEQUENCE DNA FROM REGION CONFERRING RESISTANCE FROM RESISTANT ISOLATE

